

ai

--This is a divisional of co-pending application Serial No. 09/313,123 filed May 17, 2001, which is a divisional of Serial No. 08/673,388, filed June 28, 1996, now issued (US patent 5,958,745 September 28, 1999), which is a continuation-in-part of Serial No. 08/628,039, filed April 4, 1996, now issued (US patent 5,942,660 August 24, 1999), which is a continuation-in-part of Serial No. 08/614,877, filed March 13, 1996, now issued (US patent 5,959,179 September 28, 1999).--

In the Claims

✓
Cancel claims 1-40, without prejudice.

Please add claims 41- 46 as follows:

41. A threonine deaminase protein which catalyzes the conversion of threonine to α -ketobutyrate, wherein:
- the leucine residue at amino acid position 447 is replaced with alanine, isoleucine, valine, proline, phenylalanine, tryptophan, or methionine;
 - the leucine residue at amino acid position 481 is replaced with alanine, isoleucine, valine, proline, phenylalanine, tryptophan, or methionine, or
 - the leucine residue at amino acid positions 447 and 481 are independently replaced with alanine, isoleucine, valine, proline, phenylalanine, tryptophan, or methionine.
42. A nucleic acid sequence encoding a threonine deaminase protein effective to catalyze the conversion of threonine to α -ketobutyrate, wherein:
- the encoded leucine residue at amino acid position 447 is replaced with alanine, isoleucine, valine, proline, phenylalanine, tryptophan, or methionine;
 - the encoded leucine residue at amino acid position 481 is replaced with alanine, isoleucine, valine, proline, phenylalanine, tryptophan, or methionine; or

- c. the leucine residue at amino acid positions 447 and 481 are independently replaced with alanine, isoleucine, valine, proline, phenylalanine, tryptophan, or methionine.

43. A recombinant vector comprising a nucleic acid sequence encoding a threonine deaminase protein effective to catalyze the conversion of threonine to α -ketobutyrate, wherein:

- a. the encoded leucine residue at amino acid position 447 is replaced with alanine, isoleucine, valine, proline, phenylalanine, tryptophan, or methionine;
- b. the encoded leucine residue at amino acid position 481 is replaced with alanine, isoleucine, valine, proline, phenylalanine, tryptophan, or methionine; or
- c. the leucine residue at amino acid positions 447 and 481 are independently replaced with alanine, isoleucine, valine, proline, phenylalanine, tryptophan, or methionine.

44. A recombinant host cell comprising a nucleic acid sequence encoding a threonine deaminase protein effective to catalyze the conversion of threonine to α -ketobutyrate, wherein:

- a. the encoded leucine residue at amino acid position 447 is replaced with alanine, isoleucine, valine, proline, phenylalanine, tryptophan, or methionine;
- b. the encoded leucine residue at amino acid position 481 is replaced with alanine, isoleucine, valine, proline, phenylalanine, tryptophan, or methionine; or
- c. the leucine residue at amino acid positions 447 and 481 are independently replaced with alanine, isoleucine, valine, proline, phenylalanine, tryptophan, or methionine.

45. A method of preparing recombinant host cells useful to convert threonine to α -ketobutyrate, the method comprising:

- a. selecting a host cell;
- b. transforming the selected host cell with a recombinant vector, wherein the recombinant vector comprises a nucleic acid sequence encoding a threonine deaminase protein effective to catalyze the conversion of threonine to α -ketobutyrate, wherein: